

### REMARKS

The Official Action dated October 30, 2001, has been carefully considered. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

By the present Amendment, the specification at page 5, line 3 and page 7, line 17 has been amended to add the SEQ ID NOs for the corresponding sequence listings. Claim 58 is cancelled. Claims 27, 28, 32, 53 and 55 are amended for matters of form to consistently recite "sequences" and to clarify that the methods are for mediating transgenic intramolecular recombination, that transfecting is conducted and that *six* sites or modified versions thereof that allow recombination activity are used as target sequences for beta recombinase. Claim 33 is amended to clarify the two or more intramolecular recombination events. Claims 32, 35-40, 52, 56 and 57 are amended for matters of form and/or to clarify the limitations therein, generally in accord with the Examiner's suggestions. Claims 41-44, 49 and 50 are amended to clarify the recitation of an extrachromosomal DNA substrate. Finally, claims 59 and 60 are amended to clarify the method of promoting beta recombinase activity. A Version With Markings Showing Changes Made is attached. Claims 61-63 have been added. Support for claim 61 may be found at page 8, line 29 to page 9, line 2. Support for claims 62 and 63 may be found at page 3, lines 23-27. It is believed that these changes do not involve any introduction of new matter, whereby entry is believed to be in order and is respectfully requested.

In the Official Action, the Examiner objected to the specification under 37 C.F.R. 1.821-1.825, as disclosing sequence listings which do not have a SEQ ID NO:. Accordingly, the specification has been amended to recite the SEQ ID NO's and to include a paper copy of the Sequence Listing. In addition, submitted concurrently, but under separate cover

addressed to Box Sequence Listing, are a duplicate paper copy of the Sequence Listing, a computer readable form of the Sequence Listing, and a Statement that the Sequence Listing information recorded in computer readable form is identical to the paper Sequence Listing. Therefore, the objection is traversed and it is believed that the objection has been overcome. Reconsideration is respectfully requested.

Claims 27, 28, 31-50 and 52 were objected to as being drawn to a non-elected invention on the basis that the elected invention comprised methods of genetic modification in transgenic animals. Claims 27 and 28 recite methods for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in eukaryotic cells (claim 27) or in chromatin structures of eukaryotic cells (claim 28). The methods are therefore drawn to the elected invention. Accordingly, the objection is traversed and it is believed that the objection has been overcome. Reconsideration is respectfully requested.

Claims 27, 28, 31-33, 35-50 and 52-60 were rejected under 35 U.S.C. §112, first paragraph, as being not enabled by the specification. The Examiner asserted that while the specification is enabling for a method of mediating intramolecular recombination between two *six* sites in a mouse, the specification is not enabling for all other animals because the specification fails to disclose a methodology for introducing *six* sites into a gene of interest other than through homologous recombination in an embryonic stem and embryonic stem cells exist only in mice. Specifically, the Examiner asserted that the specification does not disclose the use of appropriate vectors, specific chromatin cofactors, the specific sequences or cloning details of any eukaryotic cell in a transgenic animal, except the transgenic mouse. Finally, the Examiner asserted that while the introduction of genes are routine, the expression of the gene resulting in a particular phenotype of the animal is not routine.

However, as will be set forth in detail below, Applicants submit that the methods defined by claims 27, 28, 31-33, 35-50, 52-57, 59 and 60 are fully enabled to one of ordinary skill in the art, in accordance with the requirements of 35 U.S.C. §112, first paragraph. Accordingly this rejection is traversed and reconsideration is respectfully requested.

More particularly, according to claim 27, the invention is directed towards methods for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites or modified versions of DNA sequences located between two *six* sites in eukaryotic cells. The methods comprise the step of transfecting eukaryotic cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow the recombination activity. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to mediate recombinase activity.

According to claim 28, the invention is directed towards methods for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in chromatin structures of eukaryotic cells. The methods comprise the step of transfecting eukaryotic cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to mediate recombinase activity.

According to claim 43, the invention is directed towards methods for catalyzing site-specific resolution of DNA sequences located between *six* sites in an extrachromosomal substrate transfected into an eukaryotic cell. The methods comprise the step of catalyzing site-specific resolution with beta recombinase. The eukaryotic cell provides factors which beta recombinase is capable of using in order to mediate recombinase activity.

According to claim 53, the invention is directed towards methods for mediating transgenic intramolecular recombination in eukaryotic cells. The methods comprise the step of transfecting eukaryotic cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to mediate recombinase activity. The factors provided by eukaryotic cells comprise HMG1 chromatin-associated protein.

According to claim 55, the invention is directed towards methods for mediating transgenic intramolecular recombination in chromatin structures of eukaryotic cells. The methods comprise the step of providing eukaryotic cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow the recombination activity. The prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to mediate recombinase activity. The factors provided by the eukaryotic cells comprise HMG1 chromatin-associated protein.

Finally, according to claim 60, the invention is directed towards methods of mediating beta recombinase activity. The methods comprise the step of transfecting eukaryotic cells with beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity. The beta recombinase is capable of using eukaryotic cell factors of the eukaryotic cells to mediate recombinase activity.

Thus, the methods of claims 27, 28, 53 and 55 are directed towards meditating transgenic intramolecular recombination, i.e., recombination of sequences located in the same DNA molecule. The methods of claim 43 are directed towards catalyzing site-specific resolution, i.e., intramolecular recombination, of DNA sequences located between *six* sites in an extrachromosomal substrate, for example, a plasmid, transfected into an eukaryotic cell.

The methods of claim 60 are directed towards mediating beta recombinase activity, i.e., intramolecular recombination mediated by the beta recombinase protein.

There are various methods available and well known in the art involving the manipulation of genes in order to elucidate the relevance and function of a particular gene of interest. Examples of these techniques include site-specific recombinases such as Cre and Flp proteins, which catalyze recombination between sites located in separate DNA molecules. The methods of the present invention expand the art by using beta recombinase for mediating intramolecular recombination reactions between *six* sites in a eukaryotic cell.

Applicants note that as defined in the specification on page 3, lines 29-31, the targeted DNA sequences either contain the natural *six* sites or are modified versions of the natural *six* sites that allow recombination activity. Further, Applicants note that even though the present methods may be useful in the generation of transgenic animals, the claims recite intramolecular recombination events in eukaryotic or, more specifically, mammalian, cells, not animals. Furthermore, as the methods of the present invention are directed towards eukaryotic cells, one of ordinary skill in the art will appreciate that the methods may be used with any eukaryotic cell and any gene of interest and are not limited to the conditional knock-out or knock-in of a gene of interest in the creation of transgenic animals.

As the manipulation of genes is known to one of ordinary skill in the art, there is no evidence of record that the present methods of incorporating beta recombinase as described in the specification will not work equally as well as other gene manipulation techniques, which have commonly been employed in eukaryotic cells other than those of mice, such as pigs. For example, it is commonly known in the art that genes flanked by the loxP sites will be deleted by the Cre recombinase in mammalian cells. Therefore, one of ordinary skill in the art will appreciate that the claimed methods are suitable for use in all eukaryotic cells, without limitation to the mouse mammalian cell.

Additionally, the method of transfecting a protein, such as beta recombinase, into a eukaryotic cell is known to one of ordinary skill in the art. The specification at page 5, lines 12-20 illustrates a working example, in which the beta recombinase has been integrated at different sites of chromosomes in eukaryotic cells. Since methods for transfecting a protein into a cell are known in the art, there is no undue experimentation required to transfect beta recombinase into a eukaryotic, or more specifically, mammalian cell. Applicants note that the present methods are directed to transfecting beta recombinase into a eukaryotic cell, and that beta recombinase is capable of using chromatin cofactors provided by the eukaryotic cell to mediate recombinase activity. Since the beta recombinase is capable of using cofactors provided by the eukaryotic cell, there is no requirement to provide cofactors to the cell.

A disclosure is enabling if, from the information set forth in the specification, coupled with information known in the art, one of ordinary skill in the art could make and use the invention without undue experimentation, *United States v. Teletronics, Inc.*, 8 U.S.P.Q.2d 1217, 1224 (Fed. Cir. 1988). Moreover, every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification; rather, reasonable detail must be provided in order to enable members of the public to understand and carry out the invention. *Genetech v. Novo Nordisk, A/S*, 42 U.S.P.Q.2d 1001, 1005 (Fed. Cir. 1997). Furthermore, Applicants are not required to disclose every embodiment encompassed by their claims, even in an unpredictable art. *In re Angstadt*, 190 U.S.P.Q. 214 (CCPA 1976). One of ordinary skill in the art will appreciate that various methods are known in the art to manipulate eukaryotic cells, and specifically mammalian cells. As one of ordinary skill in the art will appreciate, when beta recombinase is isolated and specific DNA sequences containing the natural *six* sites or modified versions that allow recombination activity are identified, the disclosed and claimed methods may be conducted without limitation to the specific beta recombinase or eukaryotic cell. Finally, the specification teaches how to

determine whether eukaryotic cells provide the appropriate cofactors required for beta recombinase activity and teaches the use of specific proteins. Accordingly, the specification enables the methods of claims 27, 28, 31-33, 35-50, 52-57, 59 and 60 as required by 35 U.S.C. §112, first paragraph. It is therefore submitted that the rejection under 35 U.S.C. §112, first paragraph, has been overcome. Reconsideration is respectfully requested.

Claims 27, 28, 32, 53 and 55 were also rejected under 35 U.S.C. §112, first paragraph, as not being enabled by the specification. The Examiner asserted that the specification teaches the use of *six* sites as target sequence, but fails to disclose any other target sequence. This rejection is traversed, and reconsideration is respectfully requested.

Claims 27, 28, 53 and 55 recite transfecting eukaryotic cells with prokaryotic beta recombinase and "DNA sequences containing the natural *six* sites or modified versions that allow recombination activity", in accordance with the teachings of the specification at page 3, lines 29-31. It is therefore submitted that present claims 27, 28, 32 (dependent on claim 27), 53 and 55 are enabled by the specification under 35 U.S.C. §112, first paragraph, whereby the rejection under 35 U.S.C. §112, first paragraph has been overcome. Reconsideration is respectfully requested.

Claims 27, 28, 31-33, 35-50 and 52-60 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. With respect to Claims 27, 28, 53 and 55, the Examiner asserted that the specification teaches the use of *six* sites as target sequences, but fails to disclose any other target sequence. With respect to Claim 32, the Examiner asserted that it is unclear to what the recitation of "site-specific" intramolecular recombination refers. With respect to claims 35-40, 56 and 57, the Examiner asserted that the recitation of "promotes" is unclear in correspondence with claim 32, which only results in recombination.

With respect to claim 33, the Examiner asserted that the claim refers to "two related genes" and "different DNA sequences", but is unclear how the genes are related and how the

sequences are related to a gene. Furthermore, neither independent claim 27 nor dependent claims thereon recite that multiple target sequences are introduced or how they are introduced so that multiple genes are inactivated. With respect to claims 41-44, the Examiner asserted that the recitation of "as an extrachromosomal DNA substrate" is vague and confusing. Further, the Examiner asserted that claims 49 and 50 are confusing because it is unclear how extrachromosomal DNA would be integrated into the genome.

With respect to claim 52, the Examiner asserted that the claim is vague and confusing because claim 52 recites a method for developing transgenic mammals, while the claim it is dependent thereon, claim 27, recites a method for mediating intramolecular recombination in eukaryotic cells. In addition, the Examiner asserted that claim 52 is incomplete because the final step does not result in transgenic cells. Finally, with respect to claims 58-60, the Examiner asserted that the claims are unclear and incomplete because even providing the necessary cofactors, the method will not work unless the prokaryotic beta recombinase and *six* sites are present in the eukaryotic cell. Specifically, the Examiner asserted that since beta recombinase does not exist in eukaryotic cells it is unclear how supplying cofactors would promote beta recombinase activity.

This rejection is traversed with respect to present claims 27, 28, 31-33, 35-50 and 52-57, 59 and 60, and reconsideration is respectfully requested. More particularly, claims 27, 28, 53 and 55 recite methods of mediating transgenic intramolecular recombination and comprise the step of transfecting eukaryotic cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity. Claim 32 recites a method wherein an intramolecular recombination between two *six* sites in eukaryotic cells is obtained. Thus, these claims clearly recite the target sequences.

Claim 33 recites a method wherein two or more intramolecular recombination events involving different DNA sequences located between different *six* sites occur at the same time,

as claim 27 from which it depends is not limited to a single transgenic intramolecular recombination. Thus claim 35 is clear to one skilled in the art.

Claims 41-44, 49 and 50 recite methods wherein the recombinase activity occurs within an extrachromosomal DNA substrate. Claims 62 and 63 further clarify claims 41 and 42 by reciting that the recombinase activity occurs within an extrachromosomal DNA substrate comprising a plasmid. The specification at page 12, line 23 to page 13, line 2 discloses intramolecular recombination of chromatin-integrated target substrates as set forth in claims 49 and 50. The recitation of "within an extrachromosomal DNA substrate" is clear to one of ordinary skill in the art.

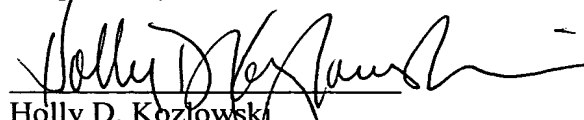
Claim 52 recites a method according to claim 27 comprising the step of selecting mammalian cells from the group consisting of eukaryotic cells, transfecting the cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity, and detecting the occurrence of an intramolecular recombination in the resulting transgenic mammalian cells. Thus, claim 52 clearly and properly depends from claim 27.

Finally, claims 59 and 60 recite methods for mediating beta recombinase activity. Claim 60, as detailed above, is directed to methods of mediating beta recombinase activity wherein the beta recombinase is capable of using eukaryotic cell factors of the eukaryotic cells to mediate recombinase activity. Further, claim 59 recites a method according to claim 60, wherein the beta recombinase is a prokaryotic beta recombinase. Additionally, claim 61 has been added to recite a method wherein the eukaryotic cell factors comprise HMG1 chromatin-associated protein. Thus, these claims recite the eukaryotic cells.

It is therefore submitted that claims 27, 28, 31-33, 35-50, 52-57, 59 and 60 are definite and the rejection under 35 U.S.C. §112, second paragraph, has been overcome. Reconsideration is respectfully requested.

It is believed that the above represents a complete response to the rejections under 35 U.S.C. §112, first and second paragraphs, and places the present application in condition for allowance. Reconsideration and an early allowance are requested.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Holly D. Kozlowski", written over a horizontal line.

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## **VERSION WITH MARKINGS SHOWING CHANGES MADE**

### **In the Specification:**

The paragraph that bridges pages 4-5 is amended as follows:

Plasmids pBT233 (23), pBT338 and pCB8 (20) and PLXSN, which [carries] carry the resistance marker for G418 (26), have been previously described. The eukaryotic expression vector pSV2 beta globin (27) was kindly provided by Dr. J. Ortin (CNB). The expression plasmid pSV beta was constructed by PCR amplification of the coding sequence for the IRAP from the plasmid pBT233. The primers, SEQ ID NO: 1 and SEQ ID NO:2, respectively, used for PCR were:

betaUP 5'-GAGAGAAAGCTTGGTTGGTTGAAAATGGCT-3' (SEQ ID NO: 1) and  
betaDO 5'-GAGAGATGATCAGTACTCATTA ACTATCCC-3' (SEQ ID NO: 2).

The paragraph at page 7, lines 15-19 is amended as follows:

The primers used for the PCR amplification of the Hirt preparations were unsuitable for the analysis of genomic DNA preparations (low  $T_m$ ). Therefore a new pair of primers, SEQ ID NO: 3 and SEQ ID NO: 4, respectively, was designed:

pBT338UP158, 5'-CCGGCTCGTATGTTGTGTGGAAT-3' (SEQ ID NO: 3) and  
pBT338DO802, 5'-TGGCGAAAGGGGGATGTGCTG-3' (SEQ ID NO: 4).

### **In the Claims:**

Claims 27, 28, 32, 33, 35-44, 49, 50, 52, 53, 55-57, 59 and 60 are amended as follows:

27. (Third Amendment) A method for mediating transgenic intramolecular recombination selected from deletions of DNA [fragments] sequences located between two *six* sites and inversions of DNA [fragments] sequences located between two *six* sites, in

eukaryotic cells, comprising the step of [providing] transfecting eukaryotic cells with prokaryotic beta recombinase and [its specific target sequences] DNA sequences containing the natural *six* sites or modified versions that allow recombination activity;

wherein the prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to [exhibit] mediate recombinase activity.

28. (Third Amendment) A method for mediating transgenic intramolecular recombination selected from deletions of DNA [fragments] sequences located between two *six* sites and inversions of DNA [fragments] sequences located between two *six* sites, in chromatin structures of eukaryotic cells, comprising the step of [providing] transfecting eukaryotic cells with prokaryotic beta recombinase and [its specific target sequences] DNA sequences containing the natural *six* sites or modified versions that allow recombination activity;

wherein the prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to [exhibit] mediate recombinase activity.

32. (Amended) A method according to claim 27, wherein [site-specific] an intramolecular recombination between two *six* sites in eukaryotic cells is obtained.

33. (Third Amendment) A method according to claim 32, wherein two or more intramolecular recombination events involving different DNA sequences located between different *six* sites occur at the same time[; and

wherein each DNA sequence is located between target sequences; whereby two related genes are inactivated].

35. (Amended) A method according to claim 32, wherein [the prokaryotic beta recombinase promotes the] an intramolecular deletion of DNA sequences located between directly oriented *six* sites [in mammalian cells] is obtained.

36. (Amended) A method according to claim 32, wherein [the prokaryotic beta recombinase promotes the] an intramolecular inversion of DNA sequences located between inverted repeated *six* sites [in mammalian cells] is obtained.

37. (Twice Amended) A method according to claim 32, wherein [the prokaryotic beta recombinase] an intramolecular deletion [promotes the deletion] of a DNA [fragment] sequence located between two directly oriented *six* sites is obtained.

38. (Third Amendment) A method according to claim 32, wherein [the prokaryotic beta recombinase promotes] an intramolecular inversion of a DNA [fragment] sequence located between two inversely oriented *six* sites is obtained.

39. (Third Amendment) A method according to claim 32, wherein [the prokaryotic beta recombinase] an intramolecular deletion [promotes deletion] of a DNA [fragment] sequence located between direct repeated DNA sequences containing *six* sites is obtained.

40. (Third Amendment) A method according to claim 32, wherein [the prokaryotic beta recombinase promotes] an intramolecular inversion of a DNA [fragment] sequence located between inverted repeated DNA sequences containing *six* sites is obtained.

41. (Amended) A method according to claim 35, wherein the specific recognition sequence is located [as] within an extrachromosomal DNA substrate.

42. (Amended) A method according to claim 36, wherein the specific recognition sequence is located [as] within an extrachromosomal DNA substrate.

43. (Twice Amended) A method for [catalysing] catalyzing site-specific resolution of DNA sequences located between six sites in an extrachromosomal [target introduced] substrate transfected into an eukaryotic cell, comprising the step of [catalysing] catalyzing the site-specific resolution with beta recombinase; wherein the eukaryotic cell provides factors which beta recombinase is capable of using in order to [exhibit] mediate recombinase activity.

44. (Amended) A method according to claim 43, wherein the extrachromosomal [target] substrate is a plasmid.

49. (Twice Amended) A method according to claim 43, wherein the six sites are integrated into [in] the genome [as] of chromatin associated structures.

50. (Twice Amended) A method according to claim 43, wherein the six sites are [integrated in the genome and] wrapped on a nucleosome at several locations.

52. (Third Amendment) A method according to claim 27, [for development of transgenic mammalian cells,] further comprising the steps of selecting [eukaryotic] mammalian cells from the group consisting of [mammalian] eukaryotic cells, transfecting the

cells with prokaryotic beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity [two *six* sites], and detecting the occurrence of an intramolecular recombination [prokaryotic beta recombinase] in the resulting transgenic mammalian cells.

53. (Twice Amended) A method for mediating transgenic intramolecular recombination in eukaryotic cells, comprising the step of [providing] transfecting eukaryotic cells with prokaryotic beta recombinase and [its specific target sequences] DNA sequences containing the natural *six* sites or modified versions that allow recombination activity;

wherein the prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to [exhibit] mediate recombinase activity; and

wherein the factors provided by the eukaryotic cells comprise HMG1 chromatin-associated protein.

55. (Twice Amended) A method for mediating transgenic intramolecular recombination in chromatin structures of eukaryotic cells, comprising the step of [providing] transfecting eukaryotic cells with prokaryotic beta recombinase and [its specific target sequences] DNA sequences containing the natural *six* sites or modified versions that allow recombination activity;

wherein the prokaryotic beta recombinase is capable of using factors provided by the eukaryotic cells in order to [exhibit] mediate recombinase activity; and

wherein the factors provided by the eukaryotic cells comprise HMG1 chromatin-associated protein.

56. (Amended) A method according to claim 28, wherein [the prokaryotic beta recombinase promotes the] an intramolecular deletion of DNA sequences located between direct repeated *six* sites in the chromatin structures is obtained.

57. (Amended) A method according to claim 28, wherein [the prokaryotic beta recombinase promotes the] an intramolecular inversion of DNA sequences located between inverted repeated *six* sites in the chromatin structures is obtained.

59. (Amended) A method according to claim [58] 60, wherein the beta recombinase is a prokaryotic beta recombinase.

60. (Twice Amended) A method of [promoting] mediating beta recombinase activity comprising the step of [providing] transfecting a eukaryotic cell with beta recombinase and DNA sequences containing the natural *six* sites or modified versions that allow recombination activity; [with eukaryotic cell factors which] wherein the beta recombinase is capable of using eukaryotic cell factors of the eukaryotic cells [in order] to [exhibit] mediate recombinase activity[;

wherein the eukaryotic cell factors comprise HMG1 chromatin-associated protein].